

IN THE CLAIMS

Please amend the claims as indicated in the following listing of claims, which replaces all previous listings of claims.

1. (currently amended) A method of transfer of a gene of interest to a product vector comprising:

a) introducing into a prokaryotic host cell which expresses a gene encoding a site-specific recombinase:

a first vector comprising:

a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon;

and

a site-specific recombination recognition site, wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

a second vector comprising:

a gene encoding a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site;

wherein said first and second vectors recombine to form a co-integrate vector comprising said double stranded origins of replication from said first vector and said second vector, and

wherein said host cell further expresses a gene encoding a Rep protein that can initiate rolling circle replication at said double stranded origins of replication in said co-integrate vector, ~~and wherein said introducing permits formation of to form~~ a product vector comprising said gene of interest interposed between said double-stranded origin of replication of said second vector and said site-specific recombination recognition site, and wherein said product vector further comprises said single-stranded origin of replication of said second vector and said gene encoding said second selectable marker, ~~said~~ wherein said product vector does not include both of said negative selectable marker and said gene encoding said first selectable marker.

2. (original) The method of claim 1, wherein said prokaryotic host cell is grown under conditions which permit said first and second vectors to recombine to form a co-integrate vector.

3. (original) The method of claim 1, wherein said product vector is isolated from said prokaryotic host cell.

4. (previously presented) A pair of vectors comprising:

(a) a first vector comprising:

a gene of interest,
a gene encoding a first selectable marker,
a double-stranded origin of replication of a rolling circle replicon; and
a site-specific recombination recognition site, wherein said gene of interest is
interposed between said double-stranded origin of replication of a rolling circle replicon
and said site-specific recombination recognition site; and

(b) a second vector comprising:

a gene encoding a negative selectable marker,
a double-stranded origin of replication of a rolling circle replicon,
a site-specific recombination recognition site,
a single-stranded origin of replication, and
a gene encoding a second selectable marker, wherein said gene encoding said
negative selectable marker is interposed between said double-stranded origin of
replication of a rolling circle replicon and said site-specific recombination recognition
site,

wherein in one or both of said first and second vectors there is no second site-
specific recombinase recognition site between said double-stranded origin of replication
and said site-specific recombinase recognition site, and wherein the site specific
recombinase recognition sites in the first and second vectors are recognized by the same
site specific recombinase.

5. (original) The vectors of claim 4, wherein said first selectable marker and said second selectable marker are different.

6. (original) The vectors of claim 4, wherein said site-specific recombinase recognition site is selected from the group consisting of: *loxP*, *loxP2*, *loxP3*, *loxP23*, *lox P511*, *lox B*, *loxC2*, *loxL*, *loxR*, *loxΔ86*, *loxΔ117*, *frt*, *dif*, λ -phage *att* sites, and Φ C31 *att* sites.

7. (previously presented) The vectors of claim 4, wherein said double-stranded origin of replication of said first vector and said double-stranded origin of replication of said second vector is the double-stranded origin of replication of the filamentous bacteriophage ϕ 1.

8. (previously presented) The vectors of claim 4, wherein said double-stranded origin of replication of said first vector and said double-stranded origin of replication of said second vector is the double-stranded origin of replication of the plasmid pKym.

9. (original) The vectors of claim 4, wherein said negative selectable marker is selected from the group consisting of: *rpsL* and *sacB*.

10. (original) The vectors of claim 4, wherein said gene encoding one of said first or second selectable markers, independently, is selected from the group consisting of: kanamycin resistance gene, the ampicillin resistance gene, the spectinomycin resistance gene, the gentamycin resistance gene, the tetracycline resistance gene, the chloramphenicol resistance gene, the streptomycin resistance gene, the *strA* gene, and the *sacB* gene.

11. (previously presented) A product vector comprising:

a gene of interest;

a double-stranded origin of replication of a rolling circle replicon

a site-specific recombination recognition site

a single-stranded origin of replication; and

a nucleic acid sequence encoding a selectable marker;

wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site.

12. (previously presented) A kit for the transfer of a gene of interest to a product vector comprising:

(a) a first vector comprising:

a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a gene encoding a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, where said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

packaging materials therefore,

wherein in one or both of said first and second vectors there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.

13. (previously presented) A kit for the transfer of a gene of interest to a product vector comprising:

(a) a first vector comprising:

a cloning site for insertion of a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said cloning site for insertion of a gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a gene encoding a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,
a single-stranded origin of replication, and
a gene encoding a second selectable marker, wherein said negative
selectable marker is interposed between said double-stranded origin of replication
of a rolling circle replicon and said site-specific recombination recognition site;
and

packaging materials therefore,

wherein in one or both of said first and second vectors there is no second site-specific
recombinase recognition site between said double-stranded origin of replication and said site-
specific recombinase recognition site.

14. (previously presented) The kit of claim 12 or 13, wherein said kit further
comprises a primary host cell which supports replication of a vector having a rolling circle
double-stranded origin of replication and which possesses a site-specific recombinase specific
for said site-specific recombination site.

15. (original) The kit of claim 12 or 13, wherein said kit further comprises a site-
specific recombinase.

16. (original) The kit of claim 14, said host cell being transfectable.

17. (previously presented) The kit of claim 14, further comprising a secondary host
cell.

18. (original) The kit of claim 12 or 13, further comprising *in vitro* recombination buffer.
19. (previously presented) A pair of vectors comprising:
- (a) a first vector comprising:
- a cloning site for insertion of a gene of interest,
 - a gene encoding a first selectable marker,
 - a double-stranded origin of replication of a rolling circle replicon; and
 - a site-specific recombination recognition site, wherein said cloning site for insertion of a gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and
- (b) a second vector comprising:
- a gene encoding a negative selectable marker,
 - a double-stranded origin of replication of a rolling circle replicon,
 - a site-specific recombination recognition site,
 - a single-stranded origin of replication, and
 - a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site,

wherein in one or both of said first and second vector there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.